

Exhibit B

to PROPOSED SECOND CONSOLIDATED AMENDED COMPLAINT



DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
1401 Rockville Pike
Rockville MD 20852-1448

JUL 30 2003

Our Reference STN: 125066/0

Howard P. Richman, D.P.M.
Biopure Corporation
11 Hurley Street
Cambridge, Massachusetts 02141

Dear Dr. Richman:

This letter is in regard to your Biologics License Application (BLA) for Hemoglobin Glutamer-250 (Bovine), also known as HBOC-201 submitted under section 351 of the Public Health Service Act.

The Center for Biologics Evaluation and Research (CBER) has completed the review of all submissions made relating to your Biologics License Application. Our review finds that the information and data submitted are inadequate for final approval action at this time based on the deficiencies outlined below. }

The deficiencies may be summarized as follows:

Monitoring of study HEM-0115**Bioresearch Monitoring Inspections:**

1. FDA inspections of fourteen clinical investigator sites at which subjects had been enrolled and treated under the HEM-0115 clinical study revealed that in addition to internal monitors, Biopure had engaged the services of several contract research organizations (CROs) and private consultants who conducted multiple monitoring visits at these clinical sites. It was also noted that these CROs and private consultants were monitoring the same sites during the same study period concurrently with internal Biopure monitors.

Please provide a detailed list of:

- a. All CROs and private consultants used to monitor the HEM-0115 study.
- b. The site(s) and time period(s) during which the internal Biopure monitors, CROs and private consultants performed specific monitoring activities.
- c. The specific obligations and responsibilities for the monitoring of clinical investigators performed by internal Biopure monitors, each CRO, and each private

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consultant including, but not limited to: completing and making changes to the case report forms (CRFs), the handling of data clarification requests, and the reporting of serious adverse events (SAEs) and adverse events (AEs).

2. FDA's November 1999 inspection of Biopure revealed there was no documentation to substantiate that the firm's internal monitors, CROs, and private consultants were trained as required by Biopure SOP CN-0029. The external contract monitors' training documentation forms (QA-0078) were found to be incomplete, and did not identify the SOPs reviewed or the trainer. There was also no documentation to ensure that external contract monitors were knowledgeable and proficient in current Biopure SOPs.
 - a. Please provide a detailed explanation as to how Biopure ensured the consistency of the monitoring activities performed by the internal Biopure monitors, the CROs and the private consultants who were monitoring the study at the various sites, especially at sites that had multiple monitors during the course of the study.
 - b. Please provide a detailed description of how Biopure instructed the internal monitors, each of the CROs and private consultants to report the results of their monitoring activities, such as the frequency and content of reports. For example (but not limited to this example), did Biopure expect the monitors to submit reports for each monitoring visit, or to submit integrated reports explaining findings during a defined time period?
3. Review of monitoring reports during FDA's November 1999 inspection of Biopure revealed the following:
 - a. Eight of ten pre-study monitoring reports covering six sites were not finalized and reviewed in accordance with Biopure's SOPs.
 - b. Five of eight initiation monitoring reports covering six sites were not finalized and reviewed in accordance with Biopure's SOPs.
 - c. Four of 28 monitoring reports covering six clinical sites were not finalized per SOPs; the date that each report was finalized was unknown for 22 out of 28 monitoring reports, and zero of 28 monitoring reports were reviewed in accordance with Biopure's SOPs.
 - d. FDA's November 2002 inspection of Biopure revealed that the internal Biopure monitors, the CROs, and the private consultants were still not submitting monitoring reports within the specified time frames, and Biopure was still not reviewing the monitoring reports in accordance with their own SOPs.

Please provide a detailed explanation or answer for the following:

- i. How Biopure ensured that the clinical investigators were conducting the HEM-0115 clinical study in accordance with the protocol requirements when

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the internal Biopure monitors, the CROs, and the private consultants were not submitting the monitoring reports as required, and Biopure was not reviewing the monitoring reports in accordance with their own SOPs.

- ii. Why BioPure failed to initiate appropriate corrective action after the November 1999 inspection to ensure that all monitoring reports were submitted and reviewed within the specified time frames.
4. Please provide a detailed explanation of the activities of GloboMax of Hanover, Maryland, in regards to data management and statistical analysis on the HEM-0115 study.

Clinical investigator issues

The FDA inspections of fourteen clinical investigator sites revealed significant deficiencies to include, but not limited to the following: (1) many SAEs and AEs not reported; (2) ineligible subjects enrolled, including subjects with known excludable medical risks; (3) not all subjects properly consented; and (4) numerous protocol required assessments either not performed or not performed within the timeframes specified by the protocol, including vital signs, hematology tests, clinical chemistry tests, urinalysis, ECGs, neurologic assessments, and physical examinations. Questions 7, 8 and 10 are examples of the significant deficiencies noted during the FDA inspections.

5. The samples for laboratory assessments for hematocrit, hemoglobin, and plasma hemoglobin were either not drawn, or were not consistently drawn immediately prior to subsequent transfusions. This deficiency was observed at numerous sites.

Please explain how Biopure can assure, without these required assessments, that additional transfusions were based on the individual needs of the subject.

6. During the inspections, a review of CRFs revealed that at least seven subjects presented with abnormal ECGs during the perioperative period. There was no record in their CRFs to document they were evaluated just prior to randomization/infusion to confirm that their abnormal ECGs did not meet the definition of acute life-threatening or significant destabilizing event.

Please explain how Biopure can ensure that these subjects were qualified or remained qualified to participate in the study without documentation of evaluation by a cardiologist that the abnormal ECGs were not clinically significant, and the subjects were cleared to participate in the study.

7. Please describe in detail how Biopure trained each of the clinical investigator sites, including the principal investigator, all sub-investigators, and study coordinators, before the sites began enrolling subjects and using the investigational product to ensure that the principal investigators, sub-investigators, and study coordinators fully understood the HEM-0115 protocol and all study requirements.

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8. The FDA inspections, and monitoring reports submitted by the internal Biopure monitors, CROs, and private consultants revealed: (1) missing source documents, (2) incomplete CRFs, (3) unsupported changes to data on the CRFs, and (4) discrepancies between the CRFs, hospital blood bank records, and drug accountability records regarding the date, number of units and volume of the investigational product that was dispensed, transfused, and returned, and (5) discrepancies regarding the date, volume, type and number of units of the control product (red blood cells, packed cells, whole blood, etc.) that was dispensed and transfused.

Please provide a detailed explanation as to how Biopure can ensure that the data in the BLA submission, in regards to the use of the investigational and/or control product, is accurate and reliable in light of the noted discrepancies. >

9. As described above, FDA inspections of the clinical sites revealed significant deficiencies throughout the course of the study. Several sites had patterns of protocol violations that continued unabated despite repeated monitoring visits that disclosed the on-going problems.
- a. Who was responsible for correcting problems and resolving the issues that were observed by the internal Biopure monitors, CROs, and consultants during the monitoring visits at the various clinical sites?
 - b. In response to the problems observed by the internal Biopure monitors, CROs, and consultants, were any of the principal investigators, sub-investigators and study coordinators re-trained? If so, what type of training was given? Who was responsible for the re-training?
 - c. Please explain in detail why Biopure failed to initiate appropriate corrective action, such as suspending enrollment of subjects at sites where serious deficiencies were noted, or terminating the study at sites where continued non-compliance to the protocol was observed during the multiple monitoring visits by the internal Biopure monitors, CROs, and private consultants.
10. Please provide a detailed explanation as to how Biopure can ensure that the safety and efficacy endpoints were met in light of the many protocol deficiencies including, but not limited to the examples described above, which were noted during the FDA inspections of these fourteen clinical sites.

How can BioPure ensure that the remaining thirty-two sites that were not inspected do not have the same types of deficiencies, and the data from these sites are reliable and accurate? >

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Study Conduct:

In the various submissions from Biopure related to BLA 125066 for HBOC-201, Biopure has used the term "softlock" to denote a number of different data management actions. In the BLA itself, submitted July 31, 2002, the term "softlock" appears in section 16.1.14 and in the description of the historical chain of events for the SEEC process. Similarly, the term "CEVA" (Clinical Event Validation and Adjudication) appears to have been used to denote different groups of people or different activities by a Data Coordinating Group.

Under section 16.1.14, on page 12 of the SEEC charter, "softlock" is described as the "resolution of all site queries for certain critical fields." This activity was supposed to occur before patient records were submitted to the SEEC for adjudication for the primary safety endpoint. CEVA Data Coordination Services were to be provided by Quintiles, Inc. The CEVA Team was to be responsible for collecting and evaluating patient safety documentation provided by investigative sites. CEVA was to be responsible for assembling safety assessment patient dossiers for all patients from all regions and for proofing each file for completeness prior to submission to the SEEC.

In the Historical Chain of Events: SEEC Process, there is an entry dated June 26, 2000 stating, "Softlock Criteria Approved (J. Burke)."

The letter dated September 24, 2002, contains following statements about "softlocking" databases and about CEVA and the Quintiles Data Management group:

- "The adjudication forms for each AE were prepared by CEVA based on the AEs in the *softlocked* AE database managed by the Quintiles Data Management group."
- "The first *softlock* of the database was completed on 22 February 2001 and the last 1st level patient dossier was submitted to adjudicators on 30 March 2001."
- "Data Management activities continued after the first database *softlock* and patients with changes in the database that would require resubmission of the dossiers to the SEEC were identified."
- "The final *softlock* of the database was completed on 29 June 2001 and the last 1st level patient dossier was resubmitted to adjudicators at that time."
- "This [final database as of June 29, 2001] *softlocked* database was also transferred to RRS."

1. Please answer the following questions:

- a. What is meant by the phrase, "softlocked database?"
- b. The abbreviation "CEVA" appears to be used in several different ways in the various submissions. What activities were encompassed by the term CEVA?

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- c. What was the relationship between the CEVA Data Coordinating Center and the Quintiles Data Management group? Did the Quintiles Data Management group provide the services collectively called CEVA?
- d. Who performed the evaluation of the source documents that were provided to the SEEC?
- e. What adjudication forms were prepared by CEVA based on the AEs in the softlocked AE database managed by Quintiles Data Management group?
- f. What were the "softlock" criteria that were approved by J. Burke on June 26, 2000?
- g. For each individual patient, what critical fields were to be resolved prior to "softlock" of the file and submission to SEEC?
- h. What role did Quintiles Data Management group play in managing the various softlocked databases and softlocked patient data?
- i. What data management activities continued after the first database softlock? Why were these activities not completed before submitting the first patient dossiers to the SEEC in November 2000?
- j. Please explain what is meant by "All post-softlock changes identified" for the June 8, 2001 entry in the historical chain of events timeline.
- k. Please explain the role and training of CEVA in collecting, assembling, and proofing patient safety documentation provided by investigative sites given that these functions had already been performed beginning in July, 2000 by a "new monitoring team."
- l. Please identify the members of the "new monitoring team" and their affiliations.
 - i. What was re-monitored by this team?
 - ii. How were the findings by this team reconciled with the later work performed by CEVA?
 - iii. How long did the re-monitoring by the new team take and when were these activities completed?
- m. What or who is AACT? What was the role of AACT?
- n. Please describe in detail the SAS listing that was approved by M. Gawryl on October 6, 2000.

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6. When 506 files were re-reviewed, did the original reviewers perform the second review? If not, why not?
7. Please explain who attended the adjudication meetings documented in the historical chain of events document and what was the purpose of the meetings.
8. In the chronology of major project developments for SEEC, you state that the first three submissions for 1st level SEEC review were submitted to SEEC before CEVA initiated site contacts for collection of source documents (November-December, 2000). The role of CEVA was to provide case report form and source documentation, which was "accurate, focused, and relevant." CEVA was also charged with resolving all site queries for certain critical fields BEFORE submission of any documents to SEEC. Please explain how the first three submissions were provided to SEEC before CEVA had contacted any sites.
9. How was the toxicity grading scale used by the investigator? Was the toxicity grading scale used during any remonitoring of the clinical data or the clinical sites?
10. Between February 26, 2001 and April 16, 2001, Biopure reviewed all source documents from the dossier file and submitted new AEs (N=1443) to CEVA for review by the SEEC. However, in the September 24, 2002 letter, you stated that Biopure did not have a direct role in providing information to the SEEC. Please explain the relationship and provide the identities of the clinical personnel contracted by Biopure to review and provide new source documents to the SEEC adjudicators for re-review. Were these personnel completely independent of Biopure? Why was this function not performed directly by CEVA, to whom this function was assigned by SEEC charter?
 - a. Please provide a detailed summary of the contractual obligations assumed by Quintiles and other data management services with regard to the "cleaning" of data for study HEM-0115.
 - b. Was the February 26, 2001 to April 16, 2001 review of all source documents the result of the July, 2000 remonitoring effort undertaken by the "new monitoring team?"
 - c. Were these adverse events also incorporated into the documentation upon which the Biopure medical review was based?
11. In the historical chain of events, three transfers of the SEEC database to Biopure are documented. The last transfer occurred on August 9, 2001, and the notation "complete data, locked" is recorded. On September 24, 2002, you stated that, "at no time did Biopure have access to nor responsibility for maintaining the database." However, in the text of the original BLA submitted July 31, 2002, on page 65 of the report of study HEM-0115, you stated that, "The database was subsequently transferred to Red River Statistics (RRS), Shreveport, LA. RRS and Biopure jointly completed data cleaning activities."

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These statements would appear to be contradictory. Please clarify and answer the following questions.

- a. Who prepared the database from the output of the SEEC deliberations?
 - b. Did Biopure have access to the databases constructed from the SAF-1 and SAF-2 forms?
 - c. Who provided the analysis datasets to Red River Statistics?
 - d. You stated that Quintiles completed data management activities for and hardlocked the CEVA database on 8/9/01 and that the hardlocked CEVA database was transferred to both Red River Statistics and Biopure on August 9, 2001. However, the historical chronology provided in the BLA indicates that two transfers of incomplete data from the SEEC database to Biopure occurred on July 12 and July 20, 2001. How were these databases used by Biopure?
 - e. In the September 17, 2002 letter submitted from Red River Statistics, it is noted that changes to an interim dataset dated June 29, 2001 were made by Red River Statistics and that these changes are memorialized in a SAS program module named ERRATA.SAS. This file contains additional raw data for adverse events and concomitant medications received after the June 29, 2001 cutoff date. Please clarify whether the second SEEC review contained the information that is contained in ERRATA.SAS.
12. In the letter of May 12, 2003, you stated that "it was always Biopure's intention to derive the secondary safety endpoints for the HEM-0115 study from the Investigator Database, not the internal scoring of the SEEC." Please note that it was always FDA's intent that all of the analyses, including the secondary analyses, be performed using the database constructed from the blinded review by the SEEC. Please note that the FDA letter to you, dated September 15, 1999, explicitly stated that:

"The review of data by the Independent Data Monitoring Committee (N.B. later renamed the SEEC) at these various time points (including at the conclusion of the study) was to have been blinded to treatment allocation. The determination of severity and seriousness of adverse events by unblinded investigators on-site was to be masked from the Independent Data Monitoring Committee. The Independent Data Monitoring Committee was to evaluate all adverse events and make an independent determination of intensity and seriousness of the adverse events, all in blinded fashion."

That letter went on to state that all serious adverse events, as determined in blinded fashion by the IDMC, would be categorized and evaluated according to the proposed statistical analysis plan. This statement did not distinguish between the primary safety analysis and the secondary safety endpoints, and the FDA letter of December 10, 1999 simply summarized the secondary safety endpoints that would be evaluated. In the September 15 and December 10, 1999 letters, FDA provided adequate documentation of

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what was expected for a regulatory submission. Omission of the recommended design features did not constitute grounds for imposing a clinical hold. On March 27, 2000 and July 7, 2000, you received letters from FDA that stated, "The statistical plan described notwithstanding, ultimate approval of your product depends on the totality of the evidence submitted from appropriately designed and conducted clinical studies including satisfactory risk: benefit outcomes." Biopure acknowledged these statements.

Since Biopure did not perform the secondary safety analyses as expected and in the manner recommended by FDA, please clarify the following points:

- a. Who performed the medical evaluation of the secondary safety endpoints for the purpose of reporting in the BLA?
 - b. Who generated the safety database for the secondary endpoints of study HEM-0115?
 - c. Who audited the safety database against source records and who monitored and reconciled the data. When was the database audited and when were the data reconciled?
 - d. What is the nature of affiliation and financial relationship to Biopure if the medical reviewers were not Biopure personnel?
 - e. Were the medical reviewers blinded to treatment assignment?
13. It is FDA's understanding that site-identified adverse events and source documents were provided to SEEC for determination of seriousness, intensity, and causality of the events, and that these, together with additional adverse events identified by each SEEC reviewer, were used to generate the SAF-1 dossier for each patient. This SAF-1 dossier was then used by each SEEC reviewer to assign a medical risk score that was recorded on SAF-2. Further, the SEEC charter stated that,

"SEEC identified adverse events: The SEEC is not charged with the task of identifying all potentially unreported adverse events; however, if during the course of reviewing a case for adjudication, a SEEC reviewer notes an unreported adverse event, this data (sic) should be captured." The charter further states that the CEVA Data Coordinating Center will review all completed adjudication CRFs for potential adjudication data discrepancies. If discrepancies are identified, a query will be issued to the reviewer who supplied the data."

"In order to proactively prevent the need to issue queries, the CEVA Data Coordinating Center has prepared a guideline for the completion of the adjudication CRFs which will be utilized by the SEEC." (This guideline included information about both the SAF-1 and SAF-2 forms.)

If it is the case that the SEEC reviewers used adverse events recorded on the SAF-1 forms in order to generate the adjudication recorded on the SAF-2 forms, and that the

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adverse events recorded on the SAF-1 forms could originate either from the on-site investigator or the SEEC reviewer, and it is also the case that the SAF-1 entries were not completely monitored and/or reconciled against source documents, then it may be concluded that the medical assessment of risk was based on a data set that did not conform with cGCPs. Your letter of May 12, 2003 in response to the clinical hold imposed on IND 10792 and also submitted to the BLA states that, "SEEC AE/SAE database was not designed or chartered to be consistent with Good Clinical Practice (GCP)". Please clarify how a database that was generated for the purpose of at least the primary safety analysis was not designed or chartered to be consistent with Good Clinical Practice.

- a. If SEEC members found additional adverse events over and above those reported from the clinical sites, how was the validity of the findings by the SEEC members determined, and were the additional, valid adverse events added to the database upon which the Biopure medical assessment was made?
14. In the May 12, 2003 letter, you stated that the SEEC AE/SAE database was not audited against source documents nor was (sic) data monitored or reconciled. Based on these statements, it would appear that the information reviewed for the secondary safety endpoints may have differed substantially from the data reviewed by the SEEC. Please clarify. Please explain why the SEEC AE/SAE database was not audited against source material.
15. In the May 12, 2003 letter, you stated that SEEC reviewers were not responsible for assessing intensity and causality of adverse events classified as non-serious. Please note that the December 10, 1999 letter from FDA clearly stated that it was FDA's intent that the IDMC evaluate all adverse events, not only those deemed serious by the on-site investigators. The specific language states, "The role of IDMC-2 is to review all adverse events in blinded fashion for each individual subject and to perform a separate determination of intensity and seriousness of the adverse events. The IDMC was to be blinded to investigator determination of intensity and seriousness when making this determination." Further, Biopure itself stated that the function of the IDMC-2 was "at the conclusion of the study, in blinded fashion, to review listings of all adverse events, whether serious or not, by individual subject, for all subjects enrolled in the study." Please comment.
16. In the letter of September 24, 2002, you stated that:

"In July of 2000, it was learned by Biopure Regulatory Management that clinical sites had inconsistently interpreted the type of adverse events that were to be recorded in the CRFs. In order to ensure the accuracy of the data, it would be necessary to re-monitor all clinical data submitted by the Investigators."

"In December of 2001, after a review of the data listings, it was discovered that not all of the Investigators had consistently applied the SAE definitions when recording data. A specially designated Biopure team conducted a complete Medical Review of all Serious

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Adverse Event (SAE) listings against the original CRFs and source documents submitted to Biopure to assure consistent classification, processing, and reporting of Serious Adverse Events....As a result of the audits, DCFs were written and signed by the Investigators to correct any data discrepancies discovered."

In the letter of May 12, 2003, you stated that:

"SEEC reviewers did not always apply serious criteria consistently and tended to classify known side effects of HBOCs (e.g., elevated liver function tests, jaundice) more seriously and conservatively. This is particularly true for the serious criteria pertaining to persistent/significant disability/incapacity and important medical events...

There was a high degree of disparity among individual SEEC reviewers: e.g., the difference in the number of SAEs for any one subject was frequently between 10 and 20."

A number of comments pertain:

- a. These various statements, taken together, suggest that the data recorded may at best be incomplete and at worst not constitute an adequate basis upon which to make a determination of safety or efficacy of HBOC-201.
- b. What criteria were applied to the remonitoring efforts that occurred after July, 2000? Please provide the Monitoring Guidelines that were used for this effort.
- c. Why did the remonitoring actions that occurred after July, 2000 not capture the inconsistencies in the classifications of SAEs?
- d. Please provide the names and affiliations of all members of all monitoring and re-monitoring teams that visited the clinical sites.
- e. The comments suggest that corrections, changes, additions, or subtractions may have been made to the databases after the so-called hardlock of August 9, 2001. Specifically, the letter of September 24, 2002 documents re-review of source documents in December 2001, and corrections that were signed by the on-site investigators. Please comment.
- f. Why did the remonitoring that occurred not clarify all the adverse events that were to be reviewed by the SEEC? Were any of the adverse events recorded by the SEEC, but not recorded by the on-site investigators, valid? If so, please explain why these adverse events were not also captured by the investigators and/or the monitoring teams sent by Biopure.

Please provide a fully detailed flow diagram and narrative description of the handling of all source documents, including but not limited to case report forms, patient chart records, etc. from the time of completion at the investigative site to the generation of the databases ultimately submitted to the BLA. (see item 1 above)

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Please provide a fully detailed flow diagram and narrative description of the generation of each database that was developed, to include, but not limited to, additions, subtractions, corrections, etc.

Please provide a fully detailed flow diagram and narrative description of all of the remonitoring efforts that occurred between the conclusion of the study and the submission of the BLA.

Please provide a detailed list of all the individuals, including affiliation and financial relationship to Biopure, who were involved in data management and database management activities for HEM-0115.

FDA reserves the right to re-evaluate the output of the SEEC adjudication and the determination that the primary safety endpoint was in fact met in HBM-0115 pending receipt of your answers to the above questions regarding study conduct and integrity of the data.

Clinical Trials

HEM-0115 (Efficacy):

1. The case report forms provided and the case tabulations and patient summaries contain numerous discrepancies, cross-outs, and unexplained deletions with regard to allogeneic transfusions given. It will be necessary for you to reconcile the case report form information against blood bank records and the patient charts in order to ensure the accuracy of these data that contribute to the assessment of the efficacy endpoints of the trial.
 - a. Please provide blood bank records, physician orders, and patient chart data to confirm all transfusions and infusions given.
 - b. Please cross-check these source documents and provide an accounting of all transfusion requests, transfusions administered, used and unused bags returned to the blood bank, etc. to ensure the accuracy of the data entered into the case tabulations and the case report forms.
 - c. Please provide complete and accurate summary data for all transfusions administered including, but not limited to the type and volume of product administered etc.

FDA reserves the right to re-evaluate the efficacy data pending receipt of this information.

2. Please provide the efficacy data for test and control groups in tabular format with each row of the table devoted to each individual patient. Each patient should have data entered on a single row and each row should contain all the information related to transfusion

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decisions and transfusion outcomes (relationship to prior trauma event, volume of blood lost, volume of fluids administered, volume of autologous blood collected and administered, time of randomization, baseline hemoglobin and hematocrit, etc.). The database should be constructed such that chronological events related to the efficacy endpoints can be read from left to right across the table. Each laboratory test entered into a column of the table should have the same unit of measure so that data analysis may be accomplished readily. The column headings should include, but are not limited to, date and type of surgery, time of surgery start and stop, total hemoglobin and hematocrit at randomization, reticulocyte counts at various time points, etc. The table should be constructed such that running time analyses may be performed. The data points entered into the table should refer to the source laboratory document, and the laboratory site (central, local, point of care) should be documented in the table.

FDA reserves the right to re-evaluate the efficacy data pending receipt of a table constructed in this manner.

3. Please provide all missing data for pre- and post-infusion total hemoglobin so that incremental increase in total hemoglobin due to administration of the product may be calculated. Significant numbers of data points appear to be missing from the case report forms. Based on the available data, the median increase in total hemoglobin following the loading dose of 60 g HBOC-201 would appear to be 0.3 g/dL rather than the expected 1.0 g/dL. Subsequent doses of 30 g HBOC-201 also appear to have increased the total hemoglobin concentration by approximately 0.2 to 0.3 g/dL. Please comment.
4. The BLA text states that dosing of HBOC-201 by 30 g dose intervals was intended to maintain plasma hemoglobin levels at safe and physiologically appropriate ranges. The clinical trial, however, did not specify what the target plasma hemoglobin level should be, and dosing decisions were based on the total hemoglobin concentration rather than a combination of RBC hemoglobin levels plus plasma hemoglobin levels or the plasma hemoglobin levels alone. Of the 317 patients treated with less than the full 300 g dose of HBOC-201, approximately 30% ultimately were transfused with allogeneic red blood cells. Most of these patients were transfused because clinicians did not appear to be able to manage their patients based on assessment of total hemoglobin levels, because the total hemoglobin levels did not increase as expected.

The dosing recommendations provided in Table 1 of the proposed package insert are not supported by clinical data from HEM-0115 or HEM-0114. Any dosing guidelines based on plasma hemoglobin levels would require confirmation in an adequately sized and adequately powered phase 3 clinical trial. In turn, support for dosing based on plasma hemoglobin levels would require sufficient phase 2 data to suggest that dosing based on such a measure is safe and likely to be efficacious. Please comment.

Given the information from the clinical trial, it is not possible to write adequate and safe dosing guidelines, and the utility of the dosing guidelines in Table 1 (using plasma hemoglobin levels) of the proposed package insert cannot be confirmed. Please comment.

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5. Many transfusions in the control red blood cell group were given back-to-back. This is particularly true for those patients who received only two units of blood. It is not clear whether this phenomenon also occurred for patients randomized to HBOC-201 who also received allogeneic red blood cells.
- a. Please provide the records for each hospital transfusion oversight committee together with the records for each site for transfusion practices for the specific surgical procedures performed. What measures are in place at each of the study sites for control of transfusion decisions?
- b. Please include information on allogeneic transfusion from each site for patients treated for the one year prior to initiation of the study and for concurrent patients who were not enrolled in the clinical trial. The report should include information about patients who underwent the specified orthopedic procedures and who did not predonate autologous red blood cells or use erythropoietin.
6. It would appear that transfusion avoidance for study HEM-0115 was largely driven by avoidance of allogeneic transfusion among those subjects who received only 60-90 g of HBOC-201. In this group, the median time to transfusion was 2.5 days. At 91-180 g of product, only 50% of subjects avoided transfusion and the median time from randomization to transfusion was approximately 4.5 days. Between 181 and 270 g of product, only 32% of subjects avoided transfusion, and the median time to transfusion was 3.3 days. At 300 g of product, 75% of patients received allogeneic red blood cells. The median time to transfusion was approximately 3 days. Patients who received HBOC-201 in any dose and who also received allogeneic red blood cells, received a median dose of 2 units (mean approximately 3 units) of red blood cells. This dose of red blood cells was comparable to or slightly higher than the dose received by patients randomized to the control group. Thus, at doses above 90 g of HBOC-201, not only were many patients exposed to the dose of HBOC-201, they also were also exposed to allogeneic blood in the amounts received by the control group. Please comment.
7. Patients in the test arm had, on average, approximately 1 g/dL lower total hemoglobin levels than did patients in the control arm for treatment days 2-6. At discharge, patients treated with HBOC-201 had hematocrits of 28 as compared to patients treated with allogeneic red blood cells who had discharge hematocrits of 31. Thus, patients treated with HBOC-201 were discharged from the hospital significantly more anemic than patients in the control group. Please comment.

Please re-analyze your efficacy database that FDA has asked you to construct and report your conclusions in your response to this letter. Your analyses might include, but should not be limited to, comparison of incremental increase in total hemoglobin levels as a result of administration of each unit of product or control, comparison of total hemoglobin levels on treatment days 2 through 6 for each group, a comparison of discharge hematocrits, review of changes in reticulocyte levels and other measures of oxygen delivery by the product, and an analysis of transfusions administered at each dose cohort of HBOC-201.

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HEM-0115 (Safety):

1. Many of the CRF Adverse Experience pages have cross-outs, deletions, additions, missing dates and/or times, or are marked "re-monitored". It will be necessary to reconcile the CRF information against hospital records in order to ensure the accuracy of data that contribute to the safety profile.
 - a. Please provide copies of source documents to substantiate all CRF Adverse Experience changes as enumerated above.
 - b. Please provide the names and affiliations of all individuals who entered or modified data entries on a CRF-by-CRF basis, and the dates and times when these changes were made.

FDA reserves the right to re-evaluate the safety data pending receipt of this information.

2. Please provide the safety data for test and control groups in tabular format. Each row should contain all the information related to the adverse event (please request a sample copy from FDA). The database should be constructed in such a way that the chronology of the adverse event can be read from left to right and that can be readily analyzed using statistical software. The column headings should include, but are not limited to, age, gender, CTM dose, time of surgery, time of treatment-emergent AE, time of first CTM infusion, time of last CTM infusion, time from 1st CTM infusion to time of treatment-emergent AE, time from last CTM infusion to time of treatment-emergent AE, total amount of CTM administered at time of treatment-emergent AE, official time/date of premature discontinuation of CTM, official reason for premature discontinuation of CTM, and time/date of 1st non-CTM transfusion.

FDA reserves the right to re-evaluate the safety data pending receipt of tables constructed in this manner. >

3. The incidence of acute myocardial infarction is different between the SEEC and FDA (based on entries by investigators in the Adverse Events pages of the CRF) databases.
 - a. Please provide FDA with copies of all source documents supplied to the SEEC regarding this issue.
 - b. Please provide copies of all source documents for all subjects with respect to troponin and CK-MB values.
 - c. Please supply tables for all CK-MB and troponin values, as outlined in question 2 (above). Note that each laboratory test entered into a column of the table should have the same unit of measure so that data analysis may be readily accomplished using statistical software.

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- d. There are numerous instances where datasets for the same subject report identical values on the same CRF page for both troponin I and T. Please explain how this occurred, which value/parameter is correct, and supply the correct CRF page for each value reported in these datasets.
 - e. Subjects 0915 and 1113 had troponin values entered by hand on the CRF page, yet the data printout with troponin values for this day is missing. Please explain the origin of these troponin values.
4. The incidence of acute renal failure (pre-specified as dialysis-dependency) is different between the SEEC (7 vs. 2) and FDA (3 vs. 1) databases.

Please provide FDA with all source documents provided to the SEEC regarding this issue and explain why there is a discrepancy between the investigator information and SEEC adjudication of the same events.
5. Laboratory measurements of amylase, AST, and ALT are deleted (or missing) for many HBOC-201 subjects.
 - a. Please explain why these values are missing or deleted, provide raw data for these missing data, identify the particular analyzer used to make the measurement, and the methodology used to obtain corrected values that reflect the presence of HBOC-201 at each corresponding study site.
 - b. Please explain why the amount of missing data for AST and ALT at treatment days 2 and 3 is much greater in the HBOC-201 arm than in the control arm during the perioperative period.
6. Progress notes for subject 2513 mention the possible necessity for renal dialysis, but omit entries on or around 9/16/1999.
 - a. Please provide source documents for all consultations and all progress, nursing, and (if applicable) dialysis nursing notes for this subject.
 - b. Similar requests pertain to subjects 4201, 4820, and 5405.
7. In your table, "Summary of Data Changes Concerning Serious Adverse Events Generated from the HEM-0115 Medical Review", which was sent to FDA after BLA submission, you list changes in adjudication and/or severity made by Biopure to the SAEs. Please provide copies of all source documents to substantiate each change.
8. Regarding product immunogenicity, please provide details of the antibody assay methodologies, including the definition of the IgG antibody unit, and the sensitivity and specificity used for positive and negative controls.

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9. Please evaluate the cross reactivity of these antibodies to human hemoglobin and describe a plan to assess the risks to patients who develop antibodies to HBOC-201 of repeat exposure to the drug.
10. Please provide an analysis of drug-drug interactions including interactions of HBOC-201 with anti-hypertensives, vasodilators, diuretics, cardiac glycosides, and colloid solutions, etc.
11. Please provide a list of, and analyze the data from, patients who received product near the end of the expiration period.

HEM-0114:

1. Please provide analyses on safety and efficacy data for patients who underwent orthopedic surgery in HEM-0114.
2. Please clarify what sponsor responsibilities were transferred to CRO(s) for HEM-0114.
3. The clinical protocol for HEM-0114 states that there is an Appendix G, "Clinical Safety Data Management: Definitions and Standards for Expedited Reporting; ICH E2A, March 1995", but only Appendices A and B are present in the BLA. Please include *all* appendices for this protocol in your submission.
4. Here were patients in the red blood cell treatment group who had neither completed six CTM infusions, nor reached the time limit for receiving CTM, but were transfused with allogeneic red blood cells considered as non-CTM. Please provide the reasons these transfusions were considered non-CTM.
5. Please provide explanations for withholding CTM infusion(s) when such infusions are "permitted".
6. Please detail the information on the patients not discharged from hospital, including their ultimate disposition.
7. Please address the issue of interference of laboratory tests in HEM-0114 (see below, clinical laboratory).
8. In the BLA, the normal ranges of the chemistry laboratory tests are given in a distinct tabulation. As the normal ranges might vary widely depending on the testing laboratory, the data in the listings for chemistry laboratory tests become incomprehensible in the absence of the normal range for each listing. Please provide the normal values for each laboratory test in the patient data listings adjacent to the test results. If central laboratory results are available, please provide this information as well.

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9. Please provide all post-baseline clinical hematology and chemistry data listings; including CPK, CK-MB and troponin, regardless of whether such data were from the treatment period or not.
10. Please provide complete information on the assays for antibodies to HBOC-201 and the data obtained.
11. Please provide an analysis of drug-drug interactions, including interactions of anti-hypertensives, vasodilators, diuretics, cardiac glycosides and colloid solutions with your product in HEM-0114.
12. Please provide all CRFs for HEM-0114, including laboratory printouts. In addition, please also provide:
 - a. Copies of source documents to substantiate all CRF Adverse Experience changes as enumerated elaborated in question 1 a (above) under HEM-0115 (Safety).
 - b. Names and affiliations of all individuals who entered or modified data entries on a CRF-by-CRF basis, and the dates and times when these changes were made.
13. Please provide tables for all treatment-emergent adverse events, as outlined in question 2 (above) under HEM-0115 (Safety).
14. Please provide a list of the subjects who were administered product near the end of the expiration period of the lot.
15. Please provide a copy of all training materials supplied to investigators, a check-off list to verify their attendance at all training sessions, and results of tests you administered to verify their understanding of GCP.

Other Clinical Studies

General Comments about the phase 2 studies:

1. Please provide all case report forms, individual patient data, laboratory printouts, and source documents to support claims of safety and efficacy for all phase 2 studies.
2. The case report forms that were provided for the phase II studies contain numerous discrepancies, cross-outs and unexplained alterations with regard to transfusions given. Accordingly, please provide explanations for missing and crossed-out data in the case reports a.) originally submitted with the phase 2 studies in this BLA and b.) all additional requested case report forms for the phase 2 studies.
3. Please provide the anesthesia records for all surgical patients in all of the phase 2 studies.

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4. For all phase 2 studies in which hemodynamic monitoring was performed using a PA catheter, please present a table that includes individual patient data listings for the following calculated hemodynamic parameters: systemic vascular resistance, oxygen delivery, oxygen consumption, and oxygen extraction ratio.
5. Please provide final study reports for all phase 2 studies conducted to support claims of efficacy and safety for your product.
6. Please provide the efficacy data for the test and control groups for all phase 2 studies in a tabular format with each row of the table devoted to each individual patient. Each patient should have data entered on a single row and each row should contain all the information related to transfusion decisions and transfusion outcomes (relationship to prior event, volume of blood lost volume of fluids administered, volume of autologous blood collected and administered, time of randomization, baseline hemoglobin and hematocrit, etc.) The database should be constructed such that chronological events related to the efficacy endpoints can be read from left to right across the table. Each laboratory test entered into a column of the table should have the same unit of measure so that data analysis may be accomplished readily. The column headings should include, but are not limited to, date and type of surgery, time of surgery, total hemoglobin at baseline and then at randomization, etc. The data points entered into the table should refer to the source laboratory document, and the laboratory site (local or central) should be documented in the table.
7. Please address the issue of laboratory interferences on laboratory tests in the phase 2 clinical trials.

Study M9990-0075:

8. Please provide the reasons for CTM transfusion and the data to support the decision to transfuse for each patient and each transfusion in this clinical trial.
9. You state the following conclusion for the above referenced study:

“ In summary, in the present study, HBOC-201 was used to treat post-operative anemia in cardiovascular surgery patients in the intensive care unit. We found that: HBOC –201 eliminated the need for postoperative allogeneic transfusion.” (Page 106). The statement is misleading. Only 34% of the patients who were randomized to the HBOC-201 group required no postoperative allogeneic RBC's. Please provide information on the number of patients who avoided allogeneic transfusion port operatively but who had been transfused intraoperatively..
10. The conclusions on page 106 also state that HBOC-201 was safe and well tolerated; however, the following adverse events were seen in this clinical trial:
 - a. Serious adverse events in HBOC-201 group had more SAE's than the RBC group: 19 vs. 4. Of the HBOC-201 SAE's, 11/19 were cardiovascular events such as ventricular

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tachycardia, atrial fibrillation, arterial desaturation, pericardial effusions, CHF, respiratory distress and dysrhythmias. In the RBC group, the SAE's consisted primarily of infections/ cellulitis (3/4) and small bowel obstruction (1/4). Please comment.

11. FDA notes that the only death in this study, patient 310, was infused with CTM#2 without having met the post-operative transfusion trigger criterion. According to protocol, the patient could be transfused with CTM if the hemoglobin was between 6.5 and 9 g/dl. In the case of patient 310, the pre-CTM #2 hemoglobin level was 9.5. Please provide an explanation for why this patient was given additional CTM for a low hemoglobin, when in fact the patient's recorded hemoglobin was above the level required for CTM infusion.
12. FDA notes that for subjects who discontinued CTM (section 12.2), most were stopped due to investigator concerns about safety (cardiorespiratory or neurological deterioration) and subjects experiencing adverse events. Please comment.

Study BR-0049-0144:

13. Study BR-0049-0144 references the following publication: "The Effects of Increased Doses of Bovine Hemoglobin on Hemodynamics and Oxygen Transport in Patients Undergoing Preoperative Hemodilution for Elective Abdominal Aortic Surgery", Kasper, et al. *Anesth Analg* 1998; 87: 284-291. The author of the publication concludes that Bovine hemoglobin in doses ranging between 55 and 97 grams of hemoglobin increased vascular resistance and decreased cardiac output in anesthetized surgical patients. Furthermore, he states that hemodilution with bovine hemoglobin in those doses ranges provided no apparent benefit over hemodilution with hydroxyethyl starch. Given the data that you provided for this study, please comment on the validity of the above statements.
14. In addition to intraoperative anesthesia records, please provide individual patient data listings for the following hemodynamic parameters: oxygen extraction ratio, systemic vascular resistance, oxygen consumption and oxygen delivery.
15. Please provide the DSMB members and charter for this study.

FDA reserves the right to review the clinical data of all phase 2 studies pending the receipt of all requested information.

General Comments on Clinical Studies

1. Please provide hospital records for all patients who received HBOC-201 on the basis of compassionate use.
2. Please provide data, which show that HBOC-201 can be administered safely at clinically relevant rates in the setting of uncontrolled surgical bleeding.

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3. Please submit Full Study Reports for studies where there are only abstracts and/or manuscripts in the BLA.

Statistics

1. It is not clear why the sample size was amended shortly before completion of study HEM-0115. Please explain whether the sample size was amended because of observations about the data collected to the point of sample size increase, if not, please explain why the sample size was increased.
2. An annual report was submitted on June 6, 2002 under BB-IND 2935 for the reporting period from February 1, 2000 through January 31, 2001. Study HEM-0115 was completed two and a half months before the end of the reporting period. However, the numbers of deaths reported in the annual report in the HBOC and RBC arms were 6 and 1 respectively, compared with 10 and 6, respectively, in the BLA submissions. Please explain the discrepancy in reporting and account for the under-reporting of deaths, considered to be serious adverse events, in the annual report.
3. In the HBOC-201 group, 5 deaths occurred among 31 patients who were older than 80 years compared to 1 death out of 26 in the RBC group. The difference is marginally significant ($p = 0.074$; one-sided Fisher's exact test). Please comment.
4. Although there is no significant difference between the two arms (10/350 vs 6/338; $p = 0.45$), the number of deaths observed in the study seems a little too high in consideration that these are elective surgeries (16/688 = 2.3%; 95% confidence interval is (1.3%, 3.7%)). Please comment on this observation in light of the mortality rate of a similar patient population with similar types of surgery from historical data.
5. Please provide the randomization code with detailed description of the randomization scheme and patient ID. Please also identify those who died.
6. Table 14.5.1 of BLA Amendment 2 also shows that there is a highly significant difference between the two arms (HBOC vs RBC: 81/350 vs 46/338; $p < 0.001$) in proportions of patients experiencing at least one SAE that resulted in death or persistent disability. Furthermore, there are 23.7% (83/350) of patients in the HBOC group who prematurely discontinued from CTM compared with 2.4% (8/338) in the RBC group.
 - a. Please justify the benefit of avoidance of allogeneic blood transfusion by administration of HBOC in light of higher proportions of patients experiencing at least one SAE that resulted in death or persistent disability and higher rate of premature discontinuation from CTM.
 - b. In connection with (a) above, please provide a 2 by 2 table defined by avoidance and SAE that resulted in death or persistent disability for the HBOC group.

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Clinical Laboratory Measurements

1. Although the BLA text states that in situations where the central laboratory(ies) was or were used, data management rules dictated use of the central laboratory data; however, it is not clear from the BLA text, the case report forms, the case tabulations, or the individual patient summaries which clinical laboratory results, i., local, central laboratory, or point-of-care, were captured in the clinical database. In addition, the hypertext links from the patient summaries take the reader to one of several different reportings of individual patient laboratory results. In some instances, the link takes the reader to the central laboratory result. In other instances, the link takes the reader to the case report forms. Some case report forms are associated with clinical laboratory printouts while many others are not. Thus, it is not possible to be sure whether the data reported in the case report forms represent local laboratory results or central laboratory results.
 - a. Please provide clear documentation for each clinical laboratory result for each site about what laboratory results have been submitted to the BLA for review.
 - b. The BLA text states that three different central laboratories, one each in the United States, Europe, and South Africa, were used for reporting of the clinical laboratory data. Since the data from these various sources are to be combined for study HEM-0115, please provide information about the clinical laboratory reference ranges, documentation of your efforts to assure that clinical data reported from each of the central laboratories were comparable such that the data could be combined for reporting purposes, the results of such studies, and a description of the instrumentation used for each laboratory assay at each of the three central laboratory sites.
2. Each hospital's laboratory was supposed to have been evaluated, and an assay limitation sheet listing all parameters available for patient management and for the study, was to have been provided to the hospital's laboratory and the investigator. In regard to these preconditions, please provide the following:
 - a. Documentation that this evaluation was performed at each site and that each investigator and each hospital laboratory was provided with the appropriate assay limitation sheet.
 - b. The assay limitation sheet for each site listing all parameters available for patient management.
 - c. The data from each clinical site and for each central laboratory regarding colorimetric and other potential interferences in assay results due to the presence of HBOC-201 in the plasma or serum samples.

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- d. The information about what backup facilities were available to ensure that parameters required for patient management and study conduct were available; and the results of studies to evaluate these back-up sites.
- e. The information about the training of and the specific procedures followed by both local laboratory and central laboratory personnel for the conduct of the various clinical studies reported in the BLA.
- f. The information about the training, the specific procedures used, and quality control methods used for all assays performed on point-of-care instruments used in the clinical studies.
- g. Clarify if clinical results reported in the BLA were corrected for assay interference by HBOC-201. Absent such information, it is not possible to review clinical laboratory data sufficiently to make accurate conclusions about the effect of the drug on patient care or patient safety.

In the tabulations and case report forms, you report that various clinical chemistry and other laboratory data have been deleted. Why were these data deleted?

3. The BLA contains articles and abstracts summarizing data regarding potential interference by HBOC-201 in clinical laboratory assays but does not contain any raw data documenting the interferences for commonly used laboratory assays, commonly used laboratory instrumentation, and commonly used point-of-care instrumentation.

- a. Please provide for review the data from studies to assess the degree of interference by HBOC-201 for commonly used clinical assays. These studies should provide information about interferences over the full range of concentrations of HBOC-201 to which patients will be exposed and should assess interference over the full range of likely analyte concentrations.

If HBOC-201 interferes with clinical measurements, please provide information characterizing the direction and magnitude of the bias associated with varying drug levels.

- b. Please provide information about point-of-care assays (including the name of the test system), which you have tested.
- c. Please provide a list of all analytes, by instrument used, for which
 - ii. there is no interference
 - iii. interference can be corrected
 - iv. no results can be obtained while HBOC-201 is still present in the circulation, above a specified threshold.

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- d. Please provide validation data supporting the use of commonly used clinical laboratory and point-of-care instruments for analyzing patient samples containing HBOC-201.
- e. Additional interference studies should be ongoing with samples drawn from study patients. The purpose of these studies is to validate other methods and analytes using real patients samples by using established methods as comparators. Patient samples should be banked for future use. Please comment.

Pharmacology/toxicology

Study (NC101):

1. Based on results from NC101 it appears that HBOC-201 delivers excessive amounts of oxygen to the tissue evaluated (left quadriceps muscle) and results in equally excessive global oxygenation and tissue oxygen extraction. Conversely, both stored and fresh blood normalizes tissue oxygenation parameters relative to pre-hemodilution values.

Please comment on the performance of stored and fresh whole blood in this model and provide a rationale for why apparent tissue over oxygenation by HBOC-201 is appropriate.
2. Under conditions of hypoxia, excess oxygen delivery inevitably will lead to oxidative cascades and tissue injury.
 - a. Please comment on the potential longer-term effects of excessive oxygen delivery apparently mediated by HBOC-201.
 - b. Please comment on the study duration and why a short-term evaluation was chosen to address a pivotal question (i.e. does HBOC-201 provide optimal tissue oxygenation?) most appropriately determined over a minimum of 24 hours. .
 - c. Please provide methHb concentrations for at least 24 hours following the final infusion of HBOC-201, tissue oxygenation parameters for at least 24 hours following the final infusion of HBOC-201 and an assessment of any tissue oxidative damage.
3. Study NC101 models a severe situation of isovolemic anemia and is intended to mimic the expected scenario for HBOC-201 utilization in clinical situations of surgical anemia related to blood loss. However, an important clinical parameter is volume status and patients generally don't require blood unless Hb falls below 6-7 g/dL. In study NC101 HES maintains the intra-vascular volume of swine.

Please comment on and provide evidence that clinically relevant HBOC-201 administration volumes provide adequate volume expansion in a clinically relevant large animal model.

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4. Measurements of tissue oxygen debt such as arterial acid base balance are progressively worsened in all groups following transfusion. This has been associated with poor prognosis in clinical cases of hemorrhagic shock and is difficult to explain in this model given the improvements in other parameters of oxygenation.

Please provide an explanation for this paradox.

5. The model presented in study NC101 and other subsequent canine models presented in the submission involves anesthetized animals. Inclusion of a conscious animal model would have been useful to assess oxygenation parameters in the absence of mechanical ventilation and anesthetic agents.

Please comment on why a conscious animal model was not used in any evaluation of tissue/global oxygenation.

Study (NC093):

6. The fact that continuous infusion of pentobarbital was used as the anesthetic may present a problem. Typically if an anesthetic is to be used in a study assessing hemodynamics one would specifically avoid pentobarbital. Pentobarbital may blunt the vasoreactivity caused by infusion of HBOC-201. Without a positive hypertensive control it would be difficult to assess the effects of the anesthetic.
- a. Please comment on why pentobarbital was used and any effect the choice of anesthetic may have had on the study outcome as related to hemodynamics and tissue oxygenation. Previous publications by your group (e.g. Lee, et al., *J. Appl. Physiol.* 79(1): 236-242, 1995.) demonstrate hypertension following Oxyglobin (earlier version of HBOC-1) administration.
 - b. While, it is understood that Oxyglobin and HBOC-201 different it is unclear from the data provided how HBOC-201 affects hemodynamics in a conscious animal model. Subsequently HBOC-201 may alter hemodynamic parameters (i.e. vasoconstriction) in conscious animals, which may go on to negatively influence tissue oxygenation. Please provide evidence from a conscious large animal model that vasoconstriction does not adversely influence tissue oxygenation following infusion of clinically relevant volumes of HBOC-201.
 - c. Also, please comment on the relevance of reporting oxygen delivery determined from cardiac output without knowing organ blood flow. Can you please confirm that the distribution of cardiac output or blood flow is optimal to specific organs following HBOC-201 infusion? If not, oxygen delivery and the values of oxygenation calculated from it become less meaningful.

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Tumor oxygenation studies:

All studies provided addressing tumor oxygenation by HBOC-201 are not relevant to the application and should not be considered in support of the concept of adequate tissue oxygen delivery in anemia. Please comment on tumor vascular similarities to that of essentially normal tissue, which would allow for a correlation of oxygen delivery within a tumor to that of normal tissue.

Population pharmacokinetic analyses

Under the pharmacokinetics portion of the label, you indicated that a weight-based dosing approach would yield a more consistent systemic exposure to HBOC-201. This approach may be anticipated to yield safer and equally efficacious clinical outcomes. However, your proposed dosing regimen is not weight-based. In your population pharmacokinetics analysis, clearance was found to increase with increasing body weight, which varied over the range of 23.4 kg to 170.1 kg (age: 8-90 years). HBOC-201 clearance as predicted by the model was 81% higher over the weight range of patients studied. Since the impact of body weight on HBOC-201 clearance may be different between pediatric and adult populations, please perform a separate analysis using pharmacokinetic data in adult population to examine the effect of body weight on HBOC-201 clearance. Furthermore, please determine whether weight-based dosing for HBOC-201 should be adopted. These determinations notwithstanding, as noted in question 4 under HEM-0115 (Efficacy), any dosing guidelines based on plasma hemoglobin levels would require confirmation in an adequately sized and adequately powered phase 3 clinical trial. In turn, support for dosing based on plasma hemoglobin levels would require sufficient phase 2 data to suggest that dosing based on such a measure is safe and likely to be efficacious. Please comment.

1. Your phase I studies indicated the dependence of elimination half-life of HBOC-201 on dose, but your population PK analysis did not include dose as a covariate.

Please include dose as a covariate in your population PK model to investigate the effect of dose on HBOC-201 clearance.

2. Your population pharmacokinetic analysis report is incomplete. It did not include the final model equation and examples of its application.

Please submit the complete study report with the results of the newly requested analyses.

Phase I pharmacokinetics studies:

1. You claim that the plasma concentration of and plasma exposure to HBOC-201 (hemoglobin) are approximately proportional to dose, but no formal proportionality analysis has been done on your pharmacokinetics data.

Please perform such an analysis using AUC vs. Dose and C_{max} vs. Dose.

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Dosing in patients with hepatic and renal impairment:

1. Your proposed indication for HBOC-201 is for the treatment of the signs and symptoms of acute anemia in adult patients undergoing orthopedic surgery. This patient population includes patients with hepatic and/or renal impairment. Elevations of serum creatinine and blood urea nitrogen were observed in patients administered HBOC-201. However, the impact of renal and hepatic impairment on HBOC-201 clearance has not been studied and the routes and mechanisms for clearance of HBOC-201 are not clearly described.

Please address these issues.

Repeat dose pharmacokinetics:

1. You have not conducted repeated dose pharmacokinetic studies using HBOC-201. Your population pharmacokinetic analysis indicated that patients who received HBOC-201 pre-operatively had, on average, a 61% lower clearance and 29% lower volume than those who did not receive HBOC-201 pre-operatively. These findings indicate that HBOC-201 involves a saturable elimination process. However, your proposed dosing regimen for HBOC-201 is up to 5 doses in 6 days, and has not characterized for its pharmacokinetics as repeated dose may lead to unanticipated accumulation of HBOC-201, it is necessary to characterize repeated dose pharmacokinetics by conducting a multiple dose pharmacokinetic study.

Please submit study protocol for our comments.

Change in mean plasma hemoglobin following infusion of HBOC-201:

1. You have provided a table with change in mean plasma hemoglobin following infusion of HBOC-201.

Please also provide median with range for change in plasma hemoglobin following infusion of HBOC-201.

Other Pharmacology/Toxicology studies

1. The first section of your proposed label (Section 2, Dosage and Administration) describes human dosing in the context of both preclinical and clinical pharmacokinetic studies. In Section 2.2, "Additional Dose," the sentence in lines 35-37 reads that an initial dose of 60 grams of HBOC-201 will result in a plasma hemoglobin concentration of approximately 1.4 g/dL, based on data from pharmacokinetic studies in animals and humans." Please delete the word "preclinical" from this language since human dosing guidance in product labeling is not predicated on animal pharmacokinetic data. Language regarding plasma

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hemoglobin levels achieved after administration of 60 g of HBOC-201 must be based on clinical data. (Please see question 4 in the review of efficacy of Study HEM-0115).

2. In Section 2.2 of the label, Table 1 entitled, "Extrapolated Dosage Guideline" is not informative because human dosing and administration needs to be based solely on human clinical data. As noted in question 4 under HEM-0115 (Efficacy), any dosing guidelines based on plasma hemoglobin levels require confirmation in an adequately sized and adequately powered phase 3 clinical trial. Human dosing based on plasma hemoglobin levels requires phase 2 data demonstrating that this approach is safe and efficacious. Please comment.
3. The language of lines 236-263 of the proposed package insert imply that the rat reproductive toxicity studies are less informative than the dog reproductive toxicity studies for predicting human developmental toxicities because of the lack of an inverted yolk sac structure in the human fetus and a longer time interval of histiotrophic nutrition in the rat compared to the dog. Although humans do not have an inverted yolk sac, and depend quite early in development on hemotrophic nutrition compared to other species, the histiotrophic process may supply the fetus with nutrients throughout gestation. Thus, FDA recommends that lines 257-263 of the proposed label be deleted and that more details are provided on the teratogenicity findings in the rat model. We also recommend that the rat teratogenicity results precede the language describing the dog reproductive toxicity studies in the label.
4. Instead of Pregnancy Category "C," FDA recommends that the product be labeled as Pregnancy Category "X" because:
 - a. The product is teratogenic in the rat
 - b. FDA does not envision a situation where use of HBOC-201 is less of a risk to a pregnant woman than red blood cells. Accordingly, please change the language of this section of the label to that stated in 21 CFR 201.57 for drugs with a pregnancy category of "X." Please comment.
5. Please refer to lines 416-421 of the proposed label. Your contention that preclinical studies suggest that Hemopure transports oxygen more effectively than red blood cells is not supported by the preclinical studies you submitted because most of the preclinical studies do not directly compare HBOC-201 treatment(s) with autologous or homologous red blood cell treatment(s). Please comment.
6. Preclinical studies with HBOC-201 in the cynomolgus model provide evidence of cardiotoxicity. The pathologic review of the studies noted that there was "a fairly strong association of HBOC-201 with heart lesions consisting of interstitial fibrosis accompanied by myocyte hypertrophy." The findings of cardiac toxicity in the Cynomolgus monkey studies should be described in detail in the HBOC- label. Please comment.

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Chemistry, Manufacturing and Controls

Herd Management Program:

1. Please explain how Animal husbandry practices (vaccines used, vaccination schedules, anthelmintic treatments, veterinary evaluation, and quarantine of new or sick animals and effective housing facilities) are being ensured and documented in the housing facilities that are providing the source herds for your product.
2. Please describe the health-screening program of source herds, including a list of agents that are screened, the frequency of testing, the proximity of test date to the slaughter date and the methodologies used for testing including positive and negative controls. In your response, please indicate whether necropsies are performed on animals that die unexpectedly at source herd farms. If so, how are results of these necropsies included in herd health assessment?
3. Please provide a description of the method for the assessment of "fallen stock" as well as for any other clinical signs of disease in animals that are presented at the abattoir for blood collection. Please explain any exception with regard to the exclusion of "fallen stock" and animals with clinical signs of diseases as source animals.

Blood Collection:

1. Please provide information on the type of dissection equipment that is used to isolate the jugular, its method of sanitization, and whether such equipment is single use or shared use among animals. Furthermore, please describe procedures used in dealing with accidental contamination by brain matter during the captive bolt process, and accidental contamination by the contents of the rumen following the hoisting of the animal.
2. For general guidance, we have previously provided you with a list of Core Cattle Diseases that the Agency uses as species-specific agents of concern when bovine-derived materials are used for human biologicals production.

Transmissible spongiform encephalopathies (TSE):

1. The performance of your TSE clearance studies provides some assurance of safety, however if TSE agents were discovered in source cattle for this product, these studies would not necessarily support release of the product. Additional studies may be requested by FDA in the future, as improved models for blood TSE infectivity are defined, when more is known about blood infectivity in bovines, and as assays are improved. Any safety claim with regard to TSE's in the package insert, must note the limitations inherent in the current validation studies including potential differences between TSE infectivity in blood of an infected animal and that of brain homogenates. Please comment.

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Process Validation:

1. Please explain the discrepancies with regard to the concentration of IgG after ultrafiltration stage I and II and that of post-final filtration (Tables 2.5.4.1.2.6 and 2.5.4.1.2.7, Page 3150) of C-500 intermediate. The data show an increased concentration of IgG after second filtration, whereas a decrease in the concentration is expected
2. Please explain the rationale for the change in IgG specification for C-800 from <15.6 ng/ml in 1997 to ≤ 25 ng/ml in 2002 (Page 3153) as an indicator or parameter for purification.
3. Please explain how the deviation with regard to elevated OxyHb was resolved in C-800 PQ studies (Table 2.5.4.1.4.5, and Table 2.5.4.1.4.6, Page 3161-2). Please provide complete deviation investigation reports with supporting data. It appears that increase in the % oxyHb has been a frequent occurrence, as demonstrated in the course of process validation. Please explain.
4. Please explain how the deviation with regard to low total Hb concentration was resolved (Table 2.5.4.1.4.11, Page 3164).
5. Please note that specification level for Boron concentration indicated for diafiltration step (DFA), as "*post-diafiltration less than pre-diafiltration result*" is unacceptable, considering that the limit of this compound may range from 600 ppm to 2.9 ppm. Please specify a numerical value for an upper acceptable limit of Boron, following diafiltration, and explain the rationale for choosing the specified limit.
6. Following diafiltration against DFC and concentration of hemoglobin, you have specified the pH of the final solution as ≤ 9.0 at 20°C (Table 2.5.4.1.4.12, Page 3165). Please explain what would be the acceptable lower limit for pH at this stage. In your response, please provide data supporting your rationale for the specification.
7. You have indicated that the bands that are detected between 66 and 22kD in SDS-PAGE analysis of Hemopure are likely to be stabilized hemoglobin subunits and not plasma protein impurities. Please provide characterization data to support your conclusion regarding the identity of these bands.
8. Your viral validation studies of the ultrafiltration step used in purification of C-500 compound was performed prior to recent changes in your process. Please provide validation data to establish the relevancy of the scaled-down model, used in this validation, to the current purification conditions indicated for this step. In addition, you have indicated that you could not successfully produce three batches of C-500 for the validation of the scaled-down ultrafiltration step. In your response please provide completed validation data for this step.

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9. Please provide validation data demonstrating that the of the 100kD Fractionation Ultrafilter from Pall Filtron Centrasette open channel filter to Pall Filtron Maxisette Filter, would not alter viral clearance capacity of the filtration step. In your evaluation please consider the following parameters: changes in flow rate, filter conditioning step and change from constant diafiltration volume process to constant retentate hemoglobin concentration process.

Product Characterization:

1. Your current measurements of oxygen dissociation equilibrium (i.e., P_{50} values) of the final product, using the Hemox-Analyzer are not based on complete oxygen saturation of hemoglobin, but rather are derived at approximately 80% saturation. Therefore these values do not reflect the actual P_{50} of the test article. Please provide corrected P_{50} values, taking into account the level of oxygen saturation in the test article. Both the corrected value of P_{50} as well as the calculated oxygen binding cooperativity (Hill coefficient) should be included in the final product specifications.

Please note that we reserve comments on "mechanism of action" of this product as described in your label until these issues are resolved.

2. The functional assays, used for the characterization of your product, are limited to measuring oxygen dissociation equilibrium parameters mentioned above. Please expand on these functional characteristics of HBOC-201 by determining the dependence of oxygen binding on pH (Bohr effect), chloride (Cl^-) and temperature.
3. Since HBOC-201 is composed of several molecular weight species, some understanding of the contributions of individual molecular species to the overall oxygen affinity (P_{50}) and cooperativity of the product should be established. Accordingly, please evaluate the contributions of individual molecular species to the overall oxygen affinity and cooperativity of the product and report these results to the BLA.
4. Your data indicated that Lot H6C014 had low total hemoglobin concentration and high P_{50} value. Please explain these results, and comment on a possible relationship between P_{50} measurement and hemoglobin concentration, including the effect of total hemoglobin and hemoglobin species such as methemoglobin (MetHb).
5. Carbonic anhydrase (CA) is the only impurity found in the C-500 and C-800 intermediates as detected by SDS-PAGE, immunoblotting and isoelectric focusing techniques. Other possible impurities, specifically red cell enzymes (i.e., superoxide dismutase and catalase) or their fractions should be detected by more sensitive methods. Accordingly, please present data about impurities from more sensitive assays such as, but not limited to immunoaffinity chromatography (see for example, Privalle et al., *Free Radic Biol Med* 28:1507, 2000)

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Stability:

1. Final product stability data indicate an apparent temperature-dependent increase in the high molecular weight fraction over time (>500 kD). As a case in point, it appears that at the 25 °C storage condition the high molecular weight fraction (>500 kD) will exceed its specification limit ($\leq 15\%$) prior to the proposed 3-yr expiration (Page 6697-6705, Lots H01C03 – H01C11).
 - a. Please explain the apparent temperature-dependent increase in the high molecular weight fraction (>500 kD) over time, and its potential impact on your proposed shelf life for the product at 25 °C.
 - b. Please provide updated stability data for final product lots manufactured in 2002 and 2001.
2. You have indicated that a two-column high performance size exclusion chromatography (HP-SEC) system (YMC-S, QT-0541) will be used to determine the molecular weight size distribution of final product instead of the one-column system (BioRad BioSil, QT-0394). Used in previous analysis.
 - a. Please provide complete validation studies for the newly developed HP-SEC method, and explain the rationale for implementing this new methodology.
 - b. You have indicated that the high molecular weight species (>500 kD) are not resolved using the newly developed two-column HP-SEC system (YMC-S). Please explain how the high molecular weight species will be measured in the final product.
 - c. Please provide data to demonstrate the comparability of molecular size determinations obtained using the one-column HP-SEC system (BioRad BioSil, QT-0394) with those obtained using the two-column HP-SEC system (YMC-S, QT-0541). In your response please indicate how the use of two different systems, over time, will effect the interpretation of the stability data with regard to the molecular size determination.
3. The final product release specification for N-acetylcysteine (0.13 – 0.22%) differs from its specification for the stability of the final product (0.02 – 0.22%). Please explain why is stability specification lower than release specification for -N-acetylcysteine.
4. Please measure the degree of autoxidation (i.e., methemoglobin formation) of final product at different temperatures (i.e., 5, 25, and 37°C) once it is removed from the inner pouch. For a given lot of the product, please compare these measurements at the beginning and at the end of the product shelf life.

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5. Please include the measurement of free iron/chelatable iron in the final product as an additional product release specification and as a stability-indicating test, to assess and monitor potential product degradation.
6. In- process testing of phospholipid content was conducted in the manufacturing of C-500 qualification lots. However, this measurement was discontinued in lots that have been manufactured subsequently. Please note that phospholipid content is considered a critical measure of purity for this intermediate, and therefore its determination should be included as a final release test for the C-500 intermediate

Package Inserts:

✓ We reserve comments on the labeling issues for the package insert until the BLA is otherwise acceptable.

no issue
Age
Contraind.
Ind:

Preapproval Inspection:

1. Inspectional issues from the February 3 through 7, 2003 and March 17 through 27, 2003 pre-approval inspection have not been resolved.

✓ You may request a meeting with CBER to discuss the above steps for approval. Please request the meeting at least 15 days prior to the proposed meeting date. Alternatively, you may choose to discuss this matter via a telephone call. Should you wish this meeting or a telephone discussion please call Mr. Franklin T. Stephenson in the Division of Blood Applications at 301-827-3524.

no new changes
at this time
prior to
approval.

Requested info. Within 10 days after the date of this letter, you are requested to take one of the following actions: (1) amend the application; (2) notify us of your intent to file an amendment or a new submission; (3) withdraw the application; or (4) request an opportunity for a hearing on the question of whether there are grounds for denying approval of the application. In the absence of any of the above responses, CBER may initiate action to deny the application.

Please note our review clock has been suspended with the issuance of this letter. Note also that any amendment should respond to all deficiencies listed and that a partial reply will not be considered for review nor will the review clock be reactivated until all deficiencies have been addressed.

Electronically

Sincerely yours,



Basil Golding, M.D.

Director,

Division of Hematology

Office of Blood Research and Review

Center for Biologics

Evaluation and Research

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